

Threatening a rubber hand that you feel is yours elicits a cortical anxiety response

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The feeling of body ownership is a fundamental aspect of self-consciousness. The underlying neural mechanisms can be studied by using the illusion where a person is made to feel that a rubber hand is his or her own hand by brushing the person's hidden real hand and synchronously brushing the artificial hand that is in full view. Here we show that threat to the rubber hand can induce a similar level of activity in the brain areas associated with anxiety and interoceptive awareness (insula and anterior cingulate cortex) as when the person's real hand is threatened. We further show that the stronger the feeling of ownership of the artificial hand, the stronger the threat-evoked neuronal responses in the areas reflecting anxiety. Furthermore, across subjects, activity in multisensory areas reflecting ownership predicted the activity in the interoceptive system when the hand was under threat. Finally, we show that there is activity in medial wall motor areas, reflecting an urge to withdraw the artificial hand when it is under threat. These findings suggest that artificial limbs can evoke the same feelings as real limbs and provide objective neurophysiological evidence that the rubber hand is fully incorporated into the body. These findings are of fundamental importance because they suggest that the feeling of body ownership is associated with changes in the interoceptive systems.

body image | emotion | fMRI | multisensory | self

The experience of the body as part of the self is a fundamental aspect of self-consciousness. Neuroscientists have recently begun to investigate the neural mechanisms underlying this sense of body ownership (1–3). This research has a strong bearing on the recent developments in applied neuroscience to integrate the human body with artificial limb devices (4–6) and raises fundamental questions about how the brain represents the boundary between the self and the environment (7, 8).

It has been shown that people can be induced to experience an artificial arm as their own arm (9). By brushing a person's hand, while it is out of their sight, and synchronously brushing a visible rubber hand, the person experiences the feeling that the rubber hand senses the touch of the brush and that this artificial hand is a part of his or her own body. This illusory feeling of body ownership is associated with activity in multisensory areas such as premotor cortex, and it has been proposed that the key mechanism for this effect relates to integration of visual, tactile, and proprioceptive information (2, 3). However, the imaging experiment of the rubber-hand illusion has hitherto depended on subjective report, and it has not been established whether the feeling of ownership generalizes to domains beyond visual and tactile perception. Thus, it is unclear whether artificial limbs support the full range of feelings evoked by real limbs.

From an evolutionary perspective, it is critical to protect one's body from physical damage and to maintain homeostasis. Bodily threat typically evokes anxiety, change in autonomic arousal, and a withdrawal tendency (10–13). Thus, arguably, for an object to fully qualify as part of one's own body, it should be treated as such by the brain's homeostatic emotional systems. A key demonstration of this would be that a physical threat to the

artificial limb evokes neuronal responses in areas related to the urge to withdraw and a feeling of anxiety. This hypothesis is supported by the finding that forceful bending of the finger of the rubber hand elicits enhanced sweating of the skin, as measured by skin conductance response, during the illusion of ownership (14). Here we used functional magnetic resonance imaging (fMRI) to provide objective neurophysiological evidence that the rubber hand is genuinely incorporated into a central representation of the body. We show that presentation of a stimulus threatening pain close to the rubber hand elicits cortical activity related to pain anticipation and anxiety, an effect related to a concurrent illusion of ownership. This finding indicates that artificial limbs, under appropriate circumstances, are fully incorporated into a bodily representation of the self.

We used an experimental set-up whereby the participant's head was tilted so that he could see the rubber hand with direct vision (2) (see Fig. 1). We applied synchronous brushing to the rubber hand and the participant's own real hand (which was out of sight) to produce the illusion of ownership of the hand. As a control, we used asynchronous brush-strokes, which do not elicit the illusion. Coincident with these two patterns of stimulation, we occasionally made brisk stabbing movements with a sharp needle toward the rubber hand, without actually touching it. By comparing brain responses associated with the needle threats during the synchronous and asynchronous brushing (see Fig. 1 and *Materials and Methods*), we could test our hypothesis that the homeostatic defense responses, such as anxiety, would be greater when participants experienced the illusion of ownership. Specifically, we predicted activity in the anterior cingulate cortex (ACC) and insula, areas associated with anticipation of pain, empathic pain, and anxiety (15–18). Because the strength of the illusion varies between individuals (1–3), we also could test the prediction that a stronger illusion is correlated with a stronger threat-related neuronal response in these areas. Our design also enables us to compare the degree of activity evoked by this manipulation with that evoked when the participant's real hand was threatened. This allowed us to evaluate the extent to which the threat was comparable for real and illusory ownership conditions.

Results

Subjective Ratings. The participants reported that the illusion of ownership was significantly stronger during the synchronous

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Abbreviations: ACC, anterior cingulate cortex; BOLD, blood-oxygenation-level-dependent; fMRI, functional MRI; SMA, supplementary motor area; pre-SMA, presupplementary motor area.

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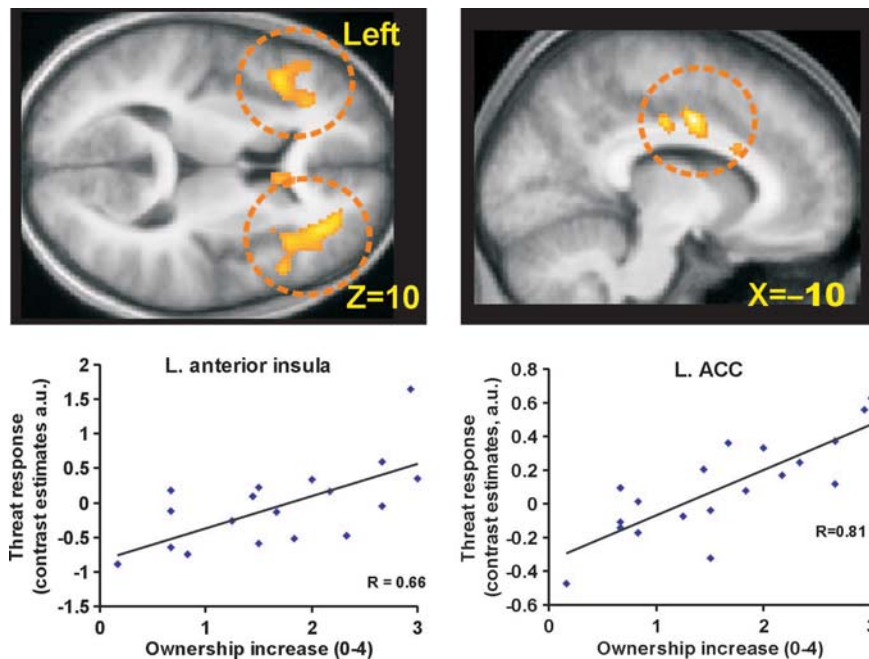


Fig. 3. Linear relationship between ownership and the anxiety responses in the bilateral anterior insula and bilateral ACC (circled). A regression analysis identified a significant relationship between the vividness ratings of the rubber-hand illusion obtained during the scans and the parameter estimates for the contrast between threat during ownership and threat during no ownership in left insula ($x = -42, y = 20, z = 10; R^2 = 0.44; P < 0.05$ corrected), right anterior insula ($x = 40, y = 28, z = 6; R^2 = 0.46, P < 0.05$ corrected; data not shown), left ACC ($x = -10, y = 4, z = 40; R^2 = 0.66, P < 0.05$ corrected), and right ACC ($x = 8, y = 4, z = 36; R^2 = 0.54; P < 0.05$ corrected; data not shown). (For all plots: Pearson's correlation, 1-tailed, $P < 0.001$; Spearman's rho, 1-tailed, $P < 0.01$.) Plots for the right side are shown in SI Fig. 10.

insular cortices probably reflected anxiety because we found a correlation between the amplitude of the threat-evoked BOLD responses in these areas during the ownership condition (as compared with the no-ownership condition) and the increase in anxiety ratings in the ownership condition (as compared with the no-ownership condition; $P < 0.05$, corrected; SI Fig. 6 and SI Table 1).

We then asked whether a relationship existed between the activity in the areas associated with ownership and those associated with anxiety. We found a significant correlation between the activity in the bilateral ventral premotor cortex that reflects the rubber-hand illusion (2) and the threat-related BOLD response in the left anterior insular cortex ($P < 0.05$, correlation analysis; see SI Fig. 7). A similar correlation was observed between the insula and left intraparietal cortex (SI Fig. 7), which is another area related to the rubber-hand illusion (2, 3). Thus, the participants who had the strongest activity in the areas related to the illusion of ownership also showed the strongest anxiety response when the rubber hand was threatened.

Next we compared the degree of activity when the rubber hand was threatened during the illusion of ownership with that expressed when the participant's real hand was threatened. We found similar levels of activity ($P > 0.05$ uncorrected) in the bilateral anterior insula (SI Fig. 8; right insula not shown), ACC (SI Fig. 8), and pre-SMA cortex (Fig. 2) when we threatened the real hand and the rubber hand in the ownership condition. Thus, these areas were active in a conjunction analysis to highlight common activation in the two contrasts: threat during ownership (of the rubber hand) and threat to real hand (see SI Fig. 8).

Discussion

We have demonstrated that when people experience that an artificial limb is part of their own body, they display the same emotional responses and defense reactions as when their real limb is threatened. The fact that similar levels of activity are evoked in

identical areas as when the person's real hand is threatened suggests that the feeling of ownership of the artificial limb is of such a degree that it fully "replaces" the real limb. Furthermore, our results reveal that the strength of the ownership-illusion (or the activity in multisensory areas reflecting this illusion) predicts the neuronal responses in areas associated with anxiety when the hand is under threat. Together these findings provide strong objective neurophysiological evidence that the rubber hand is incorporated into the body. Specifically, it shows that the physiological state of an owned artificial limb is subject to monitoring by the brain's emotional system, suggesting that artificial limbs can evoke the full range of feelings associated with real limbs.

We noted enhanced activity in medial motor areas when the rubber hand was threatened, but only when the rubber hand appeared to be part of the body. The peaks of activity lay in the SMA and pre-SMA. It is of interest that electrical stimulation of SMA in humans induces an urge to move limbs (19), and the BOLD signal in the pre-SMA increases when participants are asked to attend to the urge to produce a finger movement (20). Furthermore, activity in the SMA and pre-SMA increases before voluntary movement, reflecting the preparation to move (21–25). It is also commonly observed that medial wall motor areas are activated when humans experience or anticipate painful stimuli (16, 26–28). It has been suggested that this motor activity reflects the urge to remove the hand (27, 29) or preparation to generate an escape motor response (26). Indeed, in the present experiment, some participants spontaneously reported that they had an urge to withdraw the rubber hand in the ownership condition when the needle approached the hand. Thus, we suggest that the activity in SMA and pre-SMA in the present study reflects an urge to withdraw the hand, rather than anxiety, because this activity was not correlated with the anxiety ratings. An alternative interpretation is that this activity reflects the inhibition of the defensive motor response (30–33). Our data are consistent with both of these possibilities and indicate, whichever

is correct, that the motor system is engaged when one's own body is perceived to be under physical threat. Finally, it is unlikely that medial wall activity reflects actual movement, because the participants were not observed to move their hand or fingers and there was no reliable contraction of their index finger muscle (see *Monitoring of Hand Movement in SI Text*) and no activation of the primary motor cortex ($P > 0.001$ uncorrected).

The most compelling finding in relation to our hypothesis was that the more strongly the participants felt the rubber hand to be their own hand, the greater the activity in the ACC and left insular cortex when the hand was under threat (Fig. 3). The peaks of activation in the ACC lay in the convexity cortex of the anterior cingulate and in the cingulate sulcus. This and the nearby surrounding cortex is known to be activated during anticipation and experience of pain (16–18, 16, 34) and when seeing other people receive pain (empathy for pain) (14). We also found activation in the anterior insular cortex, and this too is known to be activated during anticipation of pain, experience of pain, and empathy for pain (15–17). Coactivation of the ACC and anterior insula in imaging experiments is strongly linked to emotional processing and interoceptive awareness (28, 35, 36). The activity in these areas probably reflected anxiety because there was a correlation between the increase in anxiety in the ownership condition and the increase in the threat-evoked BOLD responses in the ACC and insular cortex in this condition (SI Fig. 6).

Importantly, this anxiety-activity was specific to threats to one's own body and was not related to seeing the syringe or empathy for pain. Conclusive evidence for this was the direct correlation between ownership of the hand and the amplitude of the threat-evoked neuronal responses (Fig. 3). Furthermore, the participants who displayed the strongest activity in the bilateral ventral premotor cortex reflecting body ownership (2, 3) showed the strongest threat-related evoked responses in the insular cortex (SI Fig. 7). We did observe some activity in the ACC and insula that just reflected seeing the needle, as one would have expected (15) (see *SI Results in SI Text and SI Fig. 9*). However, over and above this basic activity we observed increases in anxiety-related activity during the ownership condition (SI Fig. 6), which was correlated with the ownership illusion (as described above; see Fig. 3).

The simple "subtraction" between the threat responses in the synchronous and asynchronous conditions only revealed statistical trends for activation in insula and ACC (see the BOLD plots in SI Fig. 8; $P < 0.01$, uncorrected). This probably just reflects the substantial variability between subjects in the strength of the illusion and the anxiety evoked by the needle. Indeed, it is this variability that is driving the significant correlations between the activity in insula and ACC and the subjective ratings of ownership and anxiety (described above).

Craig (35, 37) has argued that insula and ACC activity reflect engagement of an interoceptive system and that this system senses the physiological condition of the body. These areas mediate various feelings from the body such as pain, temperature, itch, and muscular sensations that are distinct from the exteroceptive systems. He further suggests that the distinction between self and nonself depends in part on the activation of this system (37) [a similar idea also was expressed by Damasio (38)]. Our results show that the feeling of ownership engendered by correlated visual, tactile, and proprioceptive information extends to engage the interoceptive system. This provides direct neurophysiological evidence for a link between the responsiveness of the ACC and insular regions and a crucial distinction between self and nonself, which supports the view that interoception is intimately linked to self perception (37).

The engagement of interoceptive emotional systems demonstrates that the effects of the self-attribution during the rubber-hand illusion generalize beyond the multisensory areas. The

natural feeling of body ownership might necessitate the engagement of the emotional systems, or the emotional responses may be a consequence of ownership mediated by multisensory mechanisms. We suggest that the recruitment of the emotional areas is an obligatory consequence of the self-attribution mediated by the multisensory mechanisms. This interoceptive activity enriches the experience of the limb as part of the body and gives it what one might call "a full sense of ownership," that is, ownership that includes homeostatic feelings such as pain, pain anticipation, and temperature. Thus, it is the engagement of the interoceptive systems that gives the artificial hand the richness of feelings that makes the illusion so eerily vivid.

Our experimental design meant that we could compare brain activity when the rubber hand or the real hand was threatened. When the participants saw the needle approaching the rubber hand during the ownership condition, they reported similar levels of anxiety as when their real hand was threatened. This relationship was mirrored by similar levels of brain activity in the insula, ACC, and medial wall motor areas in the rubber-hand-ownership condition and the real-hand condition. This verifies the strength of the illusion of ownership and suggests the engagement of common neural substrates.

It has been suggested that tools may come to be included into the body representation during extensive tool use (7). Tool use may cause changes in visuo-tactile integration (39–41) and in the receptive fields of visuo-tactile cells in the parietal cortex (42), so that it appears as if peripersonal space extends from the hand to the tool. Further, tactile sensations can be projected to the tip of hand-held tools during manipulation (43–45). However, phenomenologically, we do not experience tools as part of our own body. Thus, the detection of visuo-tactile correlations is insufficient for a body part to be fully incorporated into the body image. Our results suggest that, perhaps unlike tool use, changes in body ownership require the changes in proprioceptive and interoceptive systems. To produce the illusion that an artificial limb belongs to oneself, it is necessary to correlate tactile stimulation of the real arm and visual stimulation of the artificial limb for an extended period. This leads to a recalibration of position sense for the person's own arm (3, 9, 46, 47). This recalibration means that as the needle approaches the rubber hand, it appears to be in peripersonal space and to represent a genuine threat.

Materials and Methods

Nineteen right-handed, healthy participants took part in the imaging experiments (11 males; all aged 19–33). Participation was limited to subjects who felt the rubber-hand illusion in preliminary testing; 9 other subjects were not scanned because they did not experience the illusion (see *Pre-scanning Testing Phase in SI Text*). All subjects had given their written consent, and the study was approved by the joint National Hospital for Neurology and Neurosurgery/Institute of Neurology Ethics Committee in the U.K.

Scanning Phase. While the brain scans were being performed, the subjects rested comfortably in a supine position on the bed in the MRI scanner, with their right arm extended and placed on a support in a relaxed position (see Fig. 1). All subjects wore headphones to reduce noise and to enable them to receive auditory cues.

We used a setup where the participants could see the rubber hand with direct vision (2). Within the cylindrical head coil, the participant's head was tilted forward by $\approx 20^\circ$ by placing foam wedges beneath the head (see Fig. 1). We used a life-size rubber prosthesis of a male or female right hand (gender matched). This rubber hand was placed on a tilted ($30\text{--}45^\circ$) plastic table that was positioned over the participant's abdomen (see Fig. 1). The rubber hand was orientated in an anatomically plausible position

on the table, pointing slightly left, toward the bodily midline ($\approx 20\text{--}30^\circ$). The table was covered with a soft black material. The participant's own right hand was placed on a plastic support (30×30 cm), covered with the same soft material, which was below the table and hidden from the participant's view ($30\text{--}40$ cm away from the seen rubber hand). Finally, to reduce potential head movements, we fixed the position of the head by using foam pads.

One experimenter stood on the participant's right side so that he or she could apply brushstrokes to the participant's real right hand and the rubber hand by using small paintbrushes. This experimenter wore earphones to receive auditory cues about the onset and termination of the brushing periods. The brushstrokes were small (1 cm) and brisk (<400 ms) and applied to the upper parts of the index finger at a frequency of 1 Hz. To help the experimenter apply the same number of brushstrokes in the different conditions, he or she listened to an auditory metronome at 1 Hz over earphones and applied the stimuli with the same frequency.

The second experimenter was placed on the left side of the scanner bed. He or she held a syringe that was equipped with a sharp stainless steel needle (1.1×50 mm; Terum Europe N.V., Leuven, Belgium) (Fig. 1). The needle, which was slightly magnetic, was firmly attached to the syringe by using surgical tape, and the syringe was secured to the experimenter's arm. The experimenter could rest the hand that was holding the syringe on the table. In this position, the experimenter's hand was hidden behind a little screen on the table, 20 cm to the left of the rubber hand. The participant could not see the needle, the syringe, or the experimenter's hand when these were hidden in this way. The experimenter holding the needle wore earphones to receive auditory cues indicating when to threaten the rubber hand. When this occurred, the experimenter made brisk stabbing movements with the needle, moving it toward the index finger of the rubber hand in full view of the participant. Twelve such stabbing movements were produced in a period of 8 sec. The needle was moved close to the finger (≈ 10 mm) but not actually touching it. To minimize the risk of movement-related artefacts, both experimenters in the scanner room stood as still as possible during the scans and only made small movements of their hands to deliver the stimuli.

Four experimental runs were performed for each subject. In two of these runs, the participants were looking at the rubber hand as described above ("rubber-hand runs"; see Fig. 1 *Lower*). In two additional runs, the rubber hand was removed, and the participants looked at their own real right hand, which was placed on the table ("real-hand runs"). These two types of runs were conducted in a pseudorandomized order.

Rubber-Hand Runs. In the two experimental runs with the rubber hand, there were two main conditions (or "contexts") and short periods when we threatened the rubber hand during these contexts (see Fig. 1 *Lower*). We wanted to examine whether the needle threat evoked different neuronal responses in these different contexts (ownership or no ownership). During the ownership condition, the experimenter brushed the rubber hand and the subject's hidden hand as synchronously as possible with the two small paintbrushes. In the no-ownership condition, alternating brushstrokes were applied to the two hands. The periods of brushing the two hands synchronously or asynchronously lasted for 88 sec and were repeated 3 times in each run.

To indicate the onset of the ownership condition during the synchronous brushing, the subject was instructed to press a keypad with their left foot in a relaxed manner when they first started to feel that the rubber hand was their own (2, 3). The illusion of ownership of the rubber hand started after 14.3 ± 9.1 sec (mean \pm SD across participants). When they pressed the key they heard a brief tone over the earphones (to match the tone

presented in the no-ownership condition; see below). To control for the foot response, the participants were instructed to press the response key with their foot during the asynchronous brushing as well when they heard a tone. The timing of the presentation of these tones was yoked to the recorded times of the key response during the preceding ownership condition (Cogent 2000 software; Wellcome Department of Imaging Neuroscience, London, U.K.). After having made the responses, subjects were instructed to relax and to continue maintaining their gaze on the index finger of the rubber hand that was being brushed.

During both the ownership and no-ownership conditions, we occasionally threatened the hand with the needle. These threats were presented for periods of 8 sec (a miniblock), with periods lasting 12 sec of no threat between the threat miniblocks (Fig. 1). During each period of ownership or no-ownership condition, which lasted an average of 73.7 sec, two to four miniblocks of needle threat were presented, depending on how quickly the illusion started on that particular trial. The participants were instructed to keep looking at the index finger of the rubber hand and to avoid making any movements of their real hand throughout the experimental conditions.

After each period of synchronous or asynchronous brushing, the participants were asked to rate the following: (i) the vividness of the illusion of ownership, and (ii) the anxiety they had experienced when the hand was threatened by the needle. The participants were asked to press the foot-key 0–4 times, with 0 meaning no illusion or anxiety, respectively, and 4 meaning a very strong illusion or very strong anxiety. The participants were told to report the average feeling of ownership of the hand after the onset of the illusion (as indicated by the initial key press) and the average anxiety when the hand was being threatened. This rating procedure took 12 sec and was performed in a separate period after each period of brushing (Fig. 1). Before the next condition of synchronous or asynchronous brushing, there was a 23-sec rest period, serving as a baseline. This allowed for the illusion to disappear after the ownership condition and also gave the participants an opportunity to relax and close their eyes.

Real-Hand Runs. The real hand was threatened in separate experimental runs. The rubber hand was removed, and the participant's right hand was positioned on the table in the same place where the rubber hand was normally placed. All of the other experimental procedures were kept identical to the rubber-hand runs with two variations. First, the participant pressed the foot-key when he or she heard a tone after 14.3 sec of stimulation (matched to the onset of the illusion in the rubber-hand runs). Second, after each period of brushing, the volunteers rated the anxiety associated with the needle threat but did not have to rate the vividness of the illusion.

Acquisition and Analysis of Functional Imaging Data. The functional imaging was conducted by using a Sonata 1.5-T whole-body MRI Scanner (Siemens Medical, Erlangen, Germany) operated with a head-coil. Gradient echo T2*-weighted echo-planar images (EPIs) sensitive to the BOLD contrast were acquired as an index of local increases in synaptic activity (48) (see *EPI Scanning Parameters* in *SI Text*). A functional image volume comprised 44 continuous slices of 2-mm thickness (with a 1-mm interslice gap) covering the whole brain except the lower parts of the cerebellum [64×64 matrix, 3.0×3.0 mm in-plane resolution, Echo time (TE) = 49 msec, phase-encoding direction anterior – posterior].

The fMRI data were analyzed by using the Statistical Parametric Mapping Software 2 (SPM2; www.fil.ion.ucl.ac.uk/spm; Wellcome Department of Cognitive Neurology, London, U.K.). The functional images were realigned and unwarped to correct for head movements, coregistered with each subject's anatomical MRI, and transformed (linear and nonlinear transformation) to the space of the Montréal Neurological Institute (MNI) standard

brain. The functional images were scaled to 100 and spatially smoothed with a 10-mm full-width at half-maximum (FWHM) isotropic Gaussian kernel and smoothed in time by a 4-sec FWHM Gaussian kernel.

For each individual participant, we fitted a linear regression model (general linear model) to the data (first level analysis). For each of the experimental conditions described above, we defined one regressor for the period before the key press and one regressor for the periods after the key press. The most relevant regressors were those that were defined for the miniblocks of needle threat (hereafter referred to as “threat during ownership,” “threat during no ownership,” and “threat during real hand”). Conditions of no interest corresponding to the 12-sec periods when the participants rated the illusion and anxiety also were defined. Each condition was modeled with a boxcar function convolved with the standard SPM2 hemodynamic response function. We defined linear contrasts in the general linear model (see below). The result from this analysis was a contrast estimate for each condition for each of the 19 participants (contrast images). To accommodate intersubject variability, the contrast images from all participants were entered into a random effect group analysis (second-level analysis). One-sample *t* tests were used (18 degrees of freedom). We first used the statistical threshold of $P < 0.005$ (uncorrected) and a cluster size of 10 voxels or more to generate activations maps. For statistical inference we then used the threshold of $P < 0.05$ after a correction for the number of multiple comparisons (Family-Wise-Error correction). Because we had an *a priori* hypothesis that threatening the hand would activate certain areas associated with pain anticipation and anxiety, we used a small volume correction in these regions (15, 17, 28, 36) (see *Small Volume Correction* in *SI Text*).

Contrasts. Six main statistical analyses were performed (of which three are described in *SI Text*). First, we examined whether the

neuronal response elicited by threatening the rubber hand was modulated by the illusion of ownership, which was done by examining the contrast (threat during ownership compared with threat during no ownership).

Second, we looked for areas that showed a correlation between the strength of the rubber-hand illusion and the threat-evoked BOLD responses. Using the second-level SPM2 simple regression (correlation), we related the increase in vividness ratings of the rubber-hand illusion (ownership compared with no ownership) to the contrast estimates for the contrast (threat during ownership compared with threat during no ownership).

Third, we investigated whether the same areas were activated when we threatened the participant’s real hand as when we threatened the rubber hand during the illusion of ownership. For this, we used a conjunction analysis with the threshold of $P < 0.005$ (uncorrected) in each of the two contrasts used. This analysis, based on inclusive masking, constitutes a true logical “AND” operation (49) and identifies brain areas activated in both conditions.

Three additional analyses were performed which are described in *SI Text* (see *Additional Analyses of the fMRI Data*): (i) correlation between the activity in areas associated with ownership (premotor and parietal) and the threat-evoked activity in insula and ACC, (ii) correlations between the reported changes in anxiety and the threat-evoked BOLD responses in insula and ACC, and (iii) the main effect of threat.

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Supporting Information (SI)

Supporting Material and Methods

Pre-scanning test for rubber hand illusion

We tested potential participants on the rubber hand illusion in a separate experiment a few days before the planned brain scan. The procedure has been described in a previous paper (1), and involves the participants completing a “rubber hand questionnaire”. The nineteen who finally participated in the fMRI experiments all reported that they experienced the rubber hand illusion (see below).

For the pre-scanning test, the potential participants were seated in front of a table. A life-size, realistic, rubber prosthesis of a male or female right hand (gender matched) was placed in front of the participant. The participant’s real right hand was hidden behind a screen 20 cm to the right of the rubber hand. The rubber hand was placed in parallel with the participant’s real right hand so that it looked like the real hand. After a 60-second period of synchronous or asynchronous brushing of the rubber hand and the hidden real hand (order balanced across participants), the participants were asked to describe what they had experienced (without leading questions), and asked to complete a questionnaire containing five questions. These questions, based on those used by Botvinick and Cohen (2), required a rating of the strength of agreement or disagreement with five perceptual effects. The first two questions were relevant to the illusion (“Feeling that the rubber hand was my hand”, “Feeling of touch located on the rubber hand”) and the remaining three served as controls for suggestibility and compliance with task demands (2). The participants used a seven-point visual analogue scale to rate the extent to which these statements did or did not apply. On this scale, 1 meant “absolutely certain that it did not apply”, 4 meant “uncertain whether or not it applied”,

and 7 meant “absolutely certain that it applied”. The nineteen volunteers who went on to participate in the fMRI experiment all confirmed that they experienced the rubber hand illusion in these initial tests (i.e. scored 5-7 on the two relevant questions). They all described typical aspects of the rubber hand illusion such as “the rubber hand feels like my hand” and “the rubber hand senses the touch”. Nine participants who denied that they felt the illusion, or were uncertain about it (i.e. scored 1-4 on the relevant questions), were not scanned.

Monitoring of hand movement

The participants were trained to keep their hand and fingers still when the needle approached the rubber hand or the real hand. A video-camera filmed the participant’s real right hand throughout the experiments. Further, the experimenter who applied the brushstrokes to the hands observed the real hand closely. Potential muscular contractions of the index finger were monitored using MR-compatible electromyograms with a set of surface electrodes placed on the right first interosseus muscle (Brain Products GmbH, Munich, Germany; the data were sampled, stored and visualized on a PC using Vision Recorder and Vision Analyser software from the same company).

EPI scanning parameters

The EPI acquisition was optimized to yield the maximal BOLD sensitivity in the orbitofrontal cortex and the amygdala without significantly reducing it in the other regions of interest such as the frontal and parietal lobes (4, 5). The slices were tilted by 20 degrees (in addition to the 20 degrees the head was actually tilted in the head-coil) with respect to the axial plane and the phase-encoding polarity was chosen such that sensitivity losses due to in-plane susceptibility gradients were minimized. In addition, a

z-shim compensation gradient prepulse (-1.3 mT/m*ms) was applied prior to the EPI readout to reduce dropouts due to through-plane susceptibility gradients. One functional image volume of the brain was collected every 3.96 seconds (Repetition time (TR)=3960 ms). To minimize the image ghost, the EPI images were reconstructed using a generalized reconstruction method based on the measured EPI k-space trajectory (6).

Small volume correction

Because we had an *a priori* hypothesis that threatening the hand would activate certain areas associated with pain anticipation and anxiety, we used a small volume correction in these regions (spheres with a radius of 16mm). Specifically, we used peaks from previous studies that have shown that the insular cortex (7) (x=-39, y=-3, z=-9; x=39, y=24, z=-3), anterior insular cortex (8) (x=±40, y=26, z=10), the anterior cingulate cortex (7) (x=±6, y=3, z=45), and the medial wall motor areas (9) (x=6, y=-6, z=62; x=10, y=6, z=50) are consistently activated in pain or pain anticipation studies (9, 10). For all other regions we used the threshold of $p < 0.05$ after a correction for the number of voxels in the whole brain (Family-Wise-Error correction).

Additional analyses of the fMRI data.

In addition to the three analyses described in the Material and Methods section of the main paper we conducted three additional analyses of the data.

First, because not all participants experienced seeing the needle approaching one's hand as threatening, as indicated by low anxiety scores, we could examine whether there was a linear relationship between the increase in anxiety during the illusion and the augmented neuronal threat response. For this we used a linear regression model (the

second-level SPM 2 simple regression model) to relate the increase in anxiety as reported by the participants during the ownership condition as compared with the no ownership condition, with the stronger neuronal threat response during the illusion (*threat during ownership – threat during no ownership*). The results are presented in the Supporting Results section below and in SI Fig. 6.

Second, we examined if there was a significant relationship between activity in the premotor or parietal cortex that reflects the illusion of ownership¹, and activity in the insula that reflects anxiety. Thus we correlated the anxiety response in the insular cortex (the parameter estimates from the contrast; *threat during synchronous – threat during asynchronous*) to the illusion related activity in the bilateral ventral premotor cortex and the left intraparietal cortex (parameter estimates from the regressor corresponding to the ownership condition). For this we used the second level SPM 2 simple regression (correlation) model and searched for voxels that showed a linear relationship with the insular activity across subjects (SI Fig. 7). We restricted this analysis to spheres of 20-mm radius around the peaks taken from our previous study where we identified areas active during the rubber hand illusion (1).

Finally, we looked for areas in which there was activity when we threatened a hand regardless of whether the person experienced the ownership illusion or not. This was done by examining the main effect of needle threat across all runs (*threat during ownership + threat during no ownership + threat during real hand*). The results are presented below and in SI Fig. 9.

Anatomical localization

The anatomical localization of the activations was related to the major sulci and gyri (11), distinguishable on a mean MRI generated from the standardized anatomical MRIs from the nineteen subjects.

Supporting Results

Subjective ratings: additional analyses

There was a significant correlation between the strength of the illusion and the anxiety when the rubber hand was threatened ($p < 0.05$; SI Fig 5B). This result indicates a strong link between the feeling of ownership of the rubber hand and the degree of anxiety evoked when it is physically under threat.

Further, the participants who reported the greatest anxiety when their real hand was threatened also tended to report the greatest anxiety when the rubber hand was threatened during the illusion. In other words there was a significant correlation between the anxiety ratings during the real hand and rubber hand ownership conditions ($p < 0.05$; SI Fig 5A). These observations highlight the similarities between the experienced anxiety when the real hand and the rubber hand were threatened.

Finally, the anxiety associated with the needle-threat in the ownership condition did not habituate. The participants rated significantly greater anxiety in the illusion condition than in the control condition throughout the fMRI experiments ($p < 0.05$ paired 2-way t-test, data not shown).

Monitoring of hand movement

Although some subjects spontaneously reported an urge to remove the hand when it was threatened by the needle, we observed no actual movements of their real hand as evident from visual inspection, video recording analysis, and the inspection of the electromyograms from a index finger muscle (right first interosseus muscle).

Main effect of seeing the needle approach a hand

Finally, we identified activity related to seeing the needle approaching a hand (main effect of threat; *threat during ownership + threat during no ownership + threat during real hand*; $p < 0.05$ corrected). In this analysis we observed activation in the insula bilaterally, ACC, and medial motor areas (SI Fig. 9 and SI Table). This activity presumably reflects the visual processing of an aversive stimulus near a hand regardless of whether the person feels ownership of the hand or not. The insular clusters were centred in the anterior part of the insula and extended into the frontal operculum. The medial wall activation included a distinct peak for the pre-SMA. We also noted conspicuous activations of the bilateral superior and inferior posterior parietal cortex and in the bilateral extrastriate cortex.

Supporting Discussion

It was not surprising that we found activity in these areas when the participants saw the needle in the asynchronous condition without illusion (the main effect of threat). These responses could reflect empathy for pain as the mirror neuron theory suggests, or simply general anxiety triggered by seeing a potentially harmful object (the needle). The crucial finding, however, is that there was additional activity in the ACC, insula and pre-SMA that was specifically related to threat to one's own body (e.g. Fig 3 and Fig 2).

In a recent fMRI study, Lloyd and colleagues (12) reported enhanced parietal, frontal and occipital activation when participants saw a rubber hand being "injured" by a needle. However, in that experiment the feeling of ownership of the hand was not assessed and the occurrence of a rubber hand illusion was probably eliminated by the incongruent tactile and visual stimulation when the needle hit the rubber hand (and not

the real hand). In the present study, we also found activity in the posterior parietal cortex and extrastriate areas, and this reflected seeing the needle near the hand (see SI Table). But crucially, this activity was found irrespective of whether the person felt ownership of the hand or not. Thus, it is likely that these activations represent the visual processing of aversive objects, rather than anticipatory anxiety related specifically to one's own body or to an artificial limb during the illusion of ownership.

It could be argued that Armel and Ramachandran have already shown that forceful bending of the finger of the rubber hand elicits anxiety (13). They measured the skin conductance response of participants and reported that it was enhanced during the illusion of ownership. However, our functional imaging study extends these findings in several important ways, because skin conductance responses are unspecific and do not inform us about the activity of particular cortical areas. First, we have shown that the interoceptive system is as strongly engaged when the rubber hand is threatened in the ownership condition as when the real hand is threatened. Second, we have demonstrated that there is activity in the pre-SMA in both these conditions, suggesting the urge for the participants to withdraw the rubber hand. Third, our results reveal a direct positive relationship between the strength of the ownership illusion and the amplitude of the threat-evoked responses in the interoceptive areas. Finally, we have shown that the premotor and parietal activity that is related to the illusion correlates with the engagement of the interoceptive system.

TABLE

Anatomical region	X	Y	Z	Peak T value ($p < 0.05$ corrected)
Main effect of threatening the hand				
L. insula and frontal operculum	-52	14	-1	8.38* [†]
R. insula and frontal operculum	52	24	0	7.49* [†]
Pre-supplementary motor area	6	14	62	5.48* [†]
R. dorsal premotor cortex	40	0	56	5.94 [†]
R. superior parietal cortex	20	-58	70	5.60 [†]
L. superior parietal cortex	-34	-50	66	4.48 [†]
L. inferior parietal cortex	-60	-42	28	5.14 [†]
R. inferior parietal cortex	60	-34	42	5.30 [†]
L. extrastriate cortex	-50	-76	14	5.61 [†]
R. extrastriate cortex	52	-72	12	6.07 [†]
Interaction between threat and ownership				
Pre-supplementary motor area	-4	2	74	5.13*
Correlation between increase in anxiety and threat response				
L. insula and frontal operculum	-46	12	6	6.29* [†]
R. anterior cingulate cortex	2	10	42	3.61*
Correlation between ownership and threat response				
L. insula	-42	20	10	3.63*
R. insula	40	28	6	3.78*
L. anterior cingulate cortex	-10	-4	40	5.65*
R. anterior cingulate cortex	8	-4	36	4.48*

(^{*)} $p < 0.05$ after small volume corrections based on an *a priori* hypothesis (cluster-level; see Methods for details).

([†]) $p < 0.05$ after corrections for multiple comparisons in the whole brain (cluster-level).

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Fig. 4. The participants' ratings of the vividness (*A*) of the rubber hand illusion and the anxiety (*B*) experienced when the needle approached the hand. The error bars denotes standard deviation.

Fig. 5. (*A*) Significant correlation between the anxiety ratings for the real hand condition and rubber hand ownership condition ($P < 0.025$, Pearson's correlation, two-tailed). (*B*) Significant relationship between the vividness of the ownership and the anxiety when the rubber hand was threatened ($P < 0.0005$, Pearson's correlation, two-tailed).

Fig. 6. Relationship between the anxiety as reported by the participants and the level of activity in the left insula [with activity extending into the frontal operculum (FO)] and the anterior cingulate cortex (ACC), respectively ($P < 0.05$ corrected; encircled). Participants who reported the greatest difference in anxiety during the ownership and no ownership conditions, also showed the greatest difference in neural activity in these areas. This is illustrated in the scatter plots where the *y* axis shows the activity in the left insula or ACC (parameter estimates for the contrast threat during ownership – threat during no ownership) and the *x* axis the anxiety (anxiety rating in the illusion condition minus the no ownership condition). The activity seen in the right insula and the right inferior prefrontal cortex was not significant after correction for multiple comparisons ($P < 0.001$ uncorrected). The activity in insula showing such a correlation occurred bilaterally, with a statistical trend for activation in the right insula ($x = 36, y = 18, z = 4, t = 3.26, R^2 = 0.3847, P = 0.002$ uncorrected; not shown) in addition to the significant activation in the left ($P < 0.05$ corrected; Table). The coordinates in standard space (MNI) are indicated.

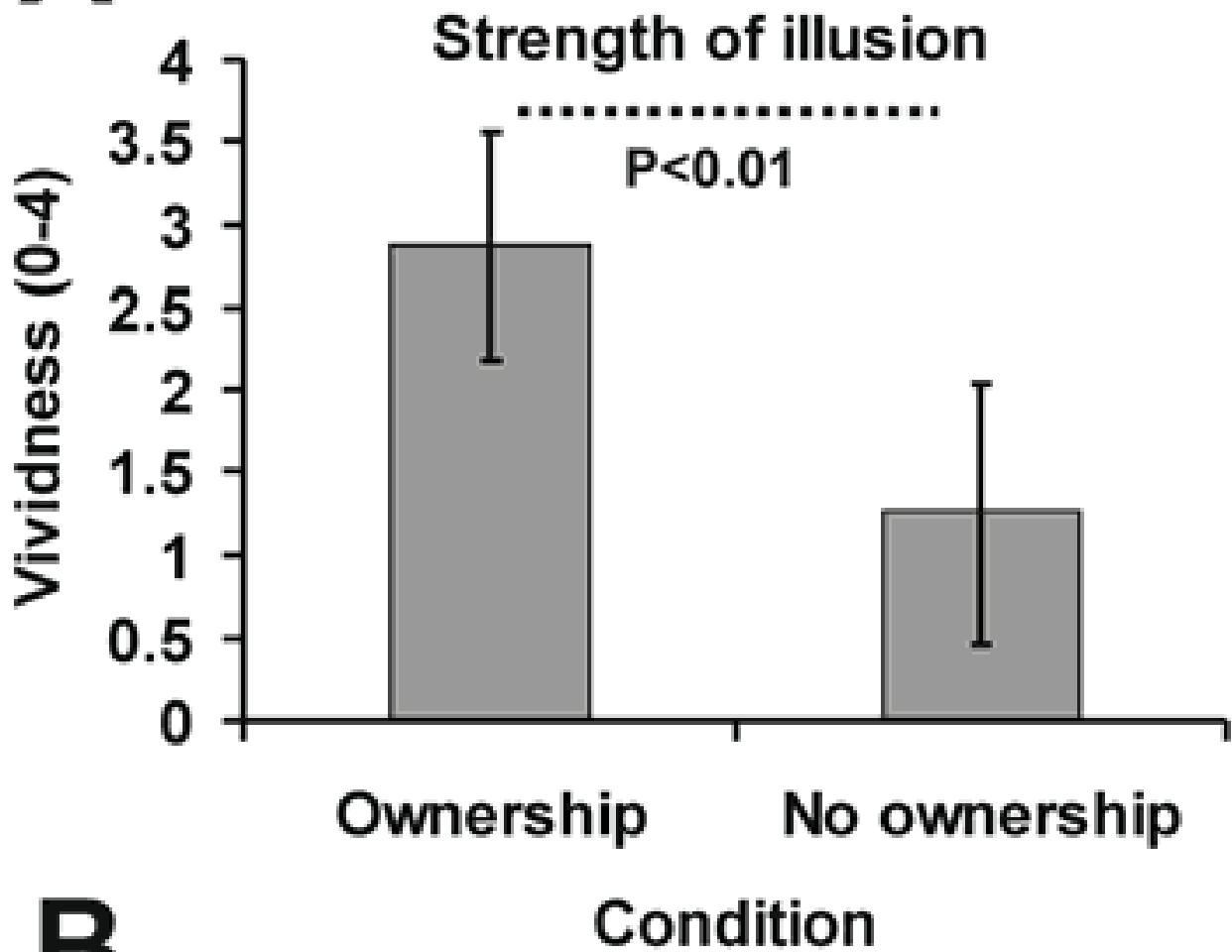
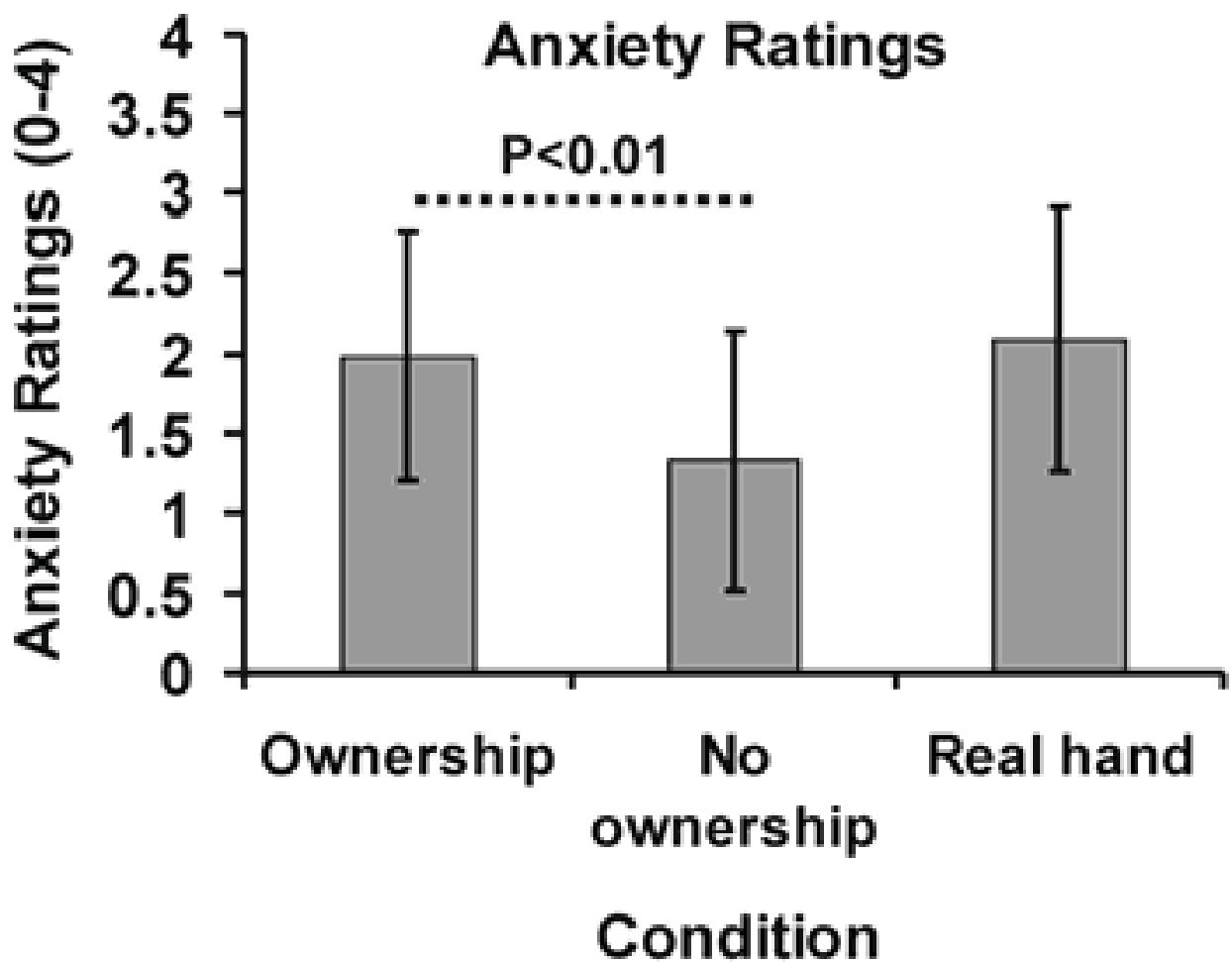
Fig. 7. Relationship between the anxiety response in the left insular cortex and the activity associated with the rubber hand illusion in the bilateral ventral premotor cortex and left posterior parietal cortex (Pearson's correlation, one-tailed, $P < 0.005$; Spearman's rho, one-tailed, $P < 0.05$). Participants who had the strongest activity in the ventral premotor cortex and the intraparietal cortex, indicating that they felt strong illusory ownership (Ehrsson *et al.* 2004), also exhibited the strongest response in the

insular cortex when the rubber hand was threatened. The insular activity was taken from the peak ($x = -46, y = 12, z = 6$; coordinates in standard space), and the coordinates for the premotor and parietal peaks are indicated (on the y axis).

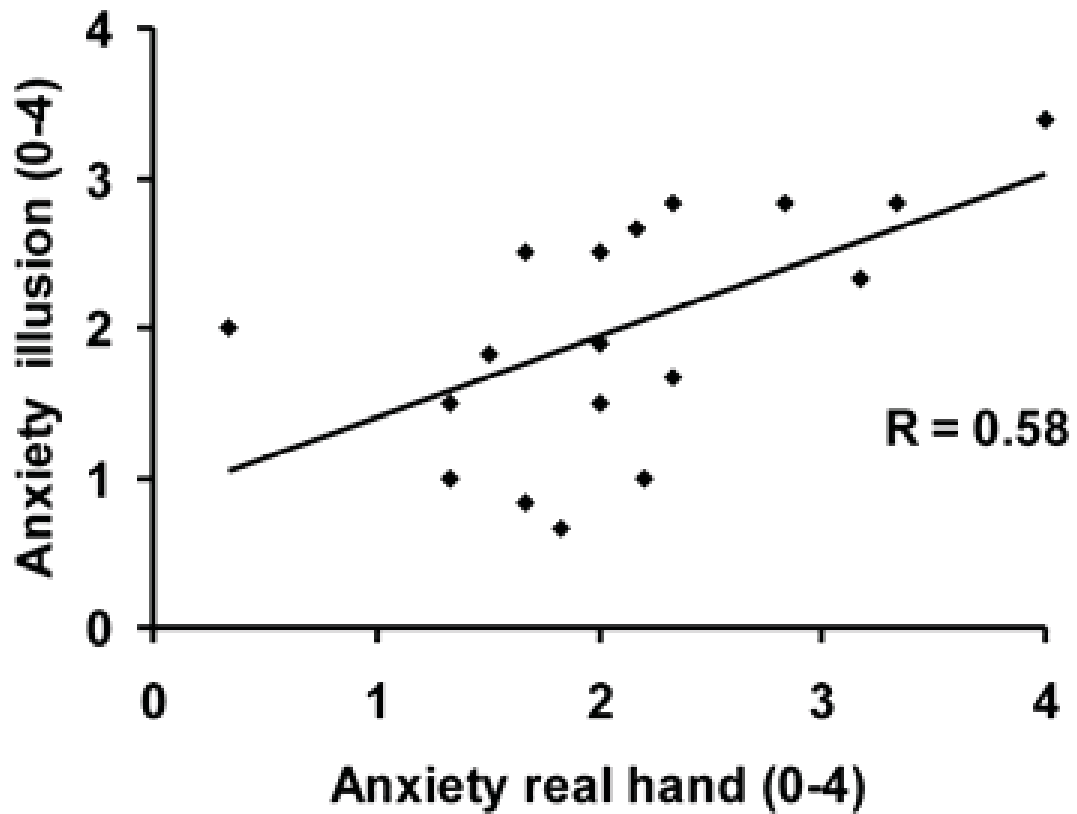
Fig. 8. Voxels in the the pre-supplementary motor area (pre-SMA, top), the anterior cingulate cortex (ACC, top, orange arrow), and the bilateral insular cortex (bottom) that were active both when the real hand was threatened and when the rubber hand was threatened during the illusion of ownership. A conjunction analysis was used where the p -values indicate the uncorrected P values for each of the two statistical contrasts (threat during ownership AND threat during real hand). We also plotted the parameter estimates from the left ACC (arrow) and the left insula (circled) (the error bars indicate standard error; for the corresponding plot of the pre-SMA activity see Fig. 2). The coordinates in standard space are indicated.

Fig. 9. Main effect of needle threat. Activation of the bilateral insula (top), presupplementary motor area (preSMA; lower), and anterior cingulate cortex (ACC; lower) was observed when the participants saw a needle approaching the hand, regardless of whether they felt ownership of it or not ($P < 0.05$ corrected; threat during ownership + threat during no ownership + threat during real hand). The laterality is indicated, as well as the coordinate in standard space.

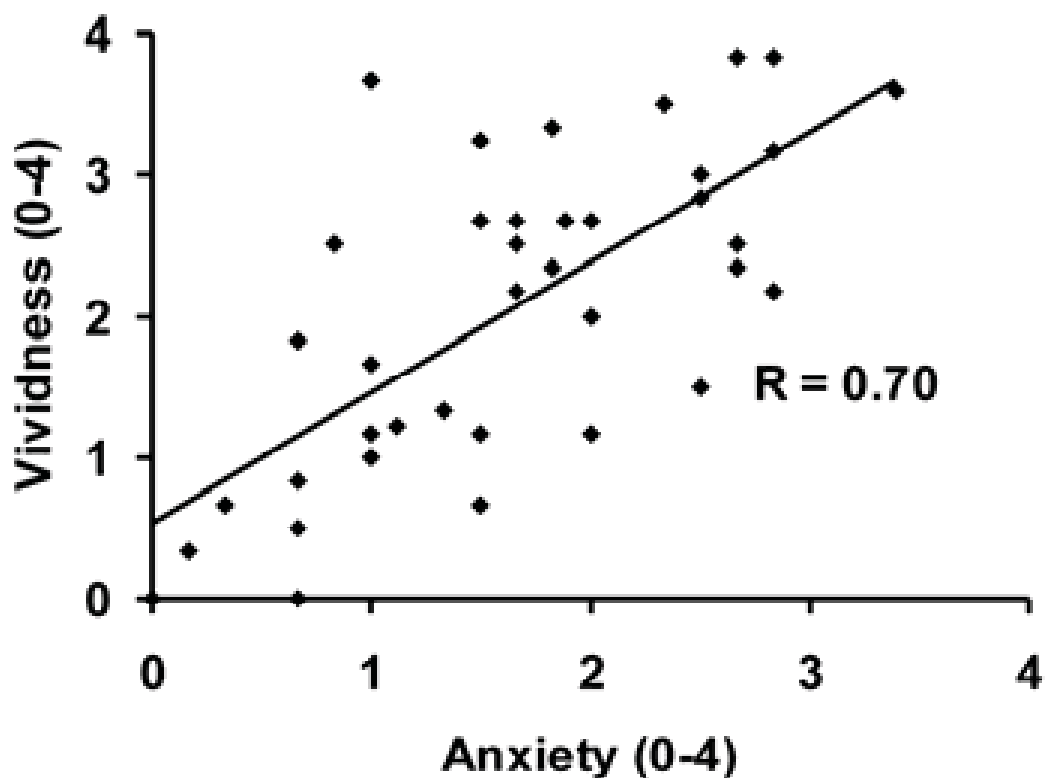
Fig. 10. Linear relationship between subjective ownership ratings and the neural anxiety responses in the right anterior insula ($x = 40, y = 28, z = 6$) and right ACC ($x = 8, y = -4, z = 36$). The plots for the left insula and left ACC are shown in Fig. 3. See the legend of Fig. 3 for details about the analysis.

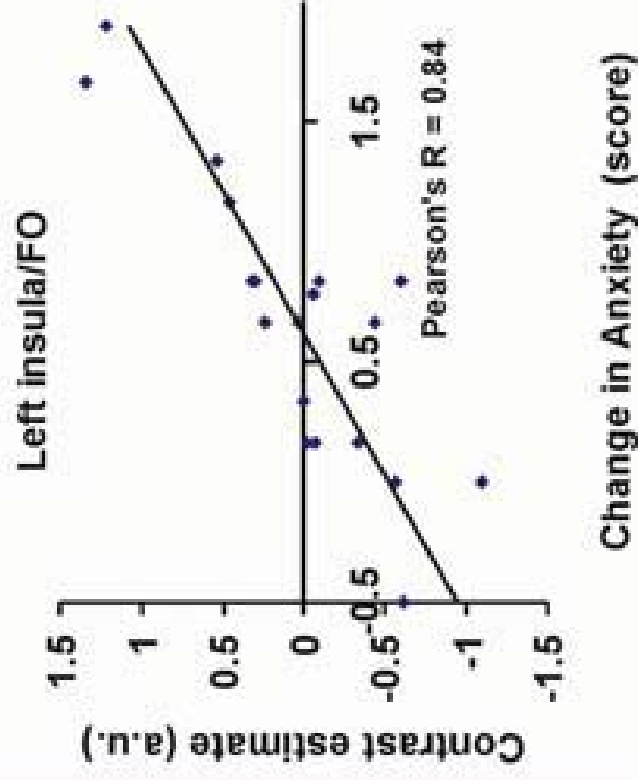
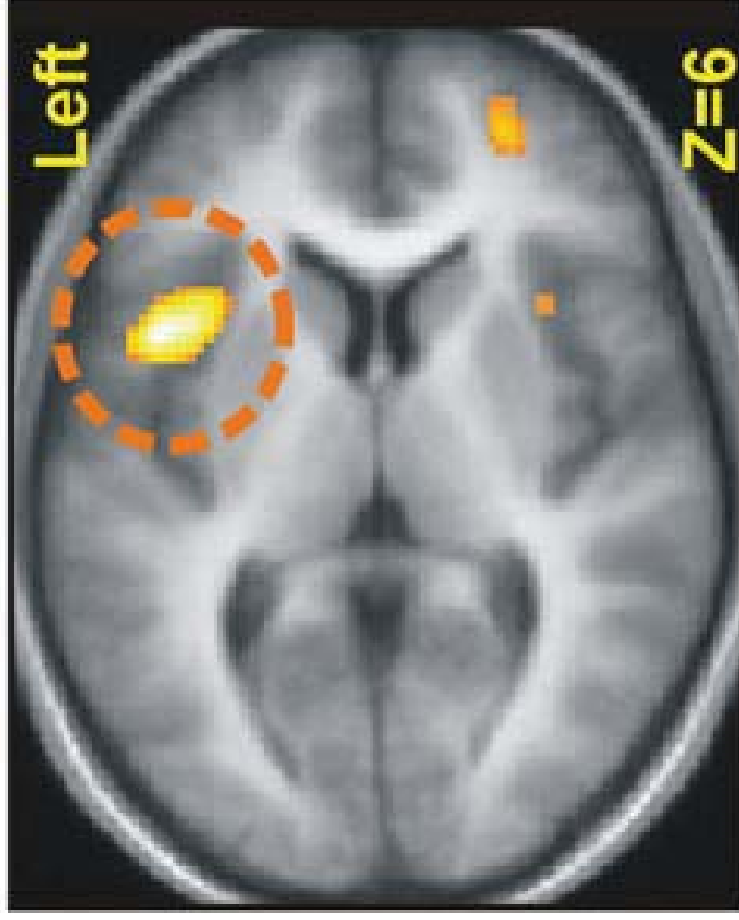
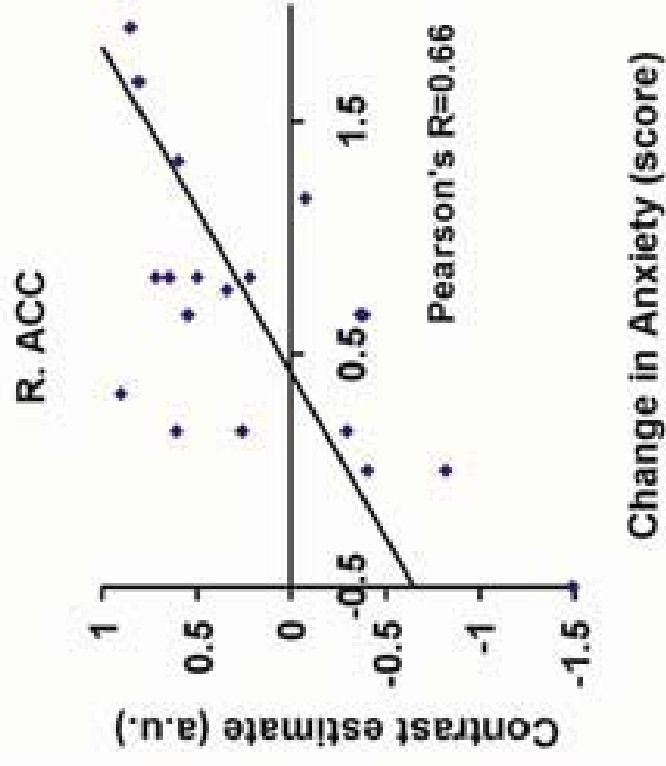
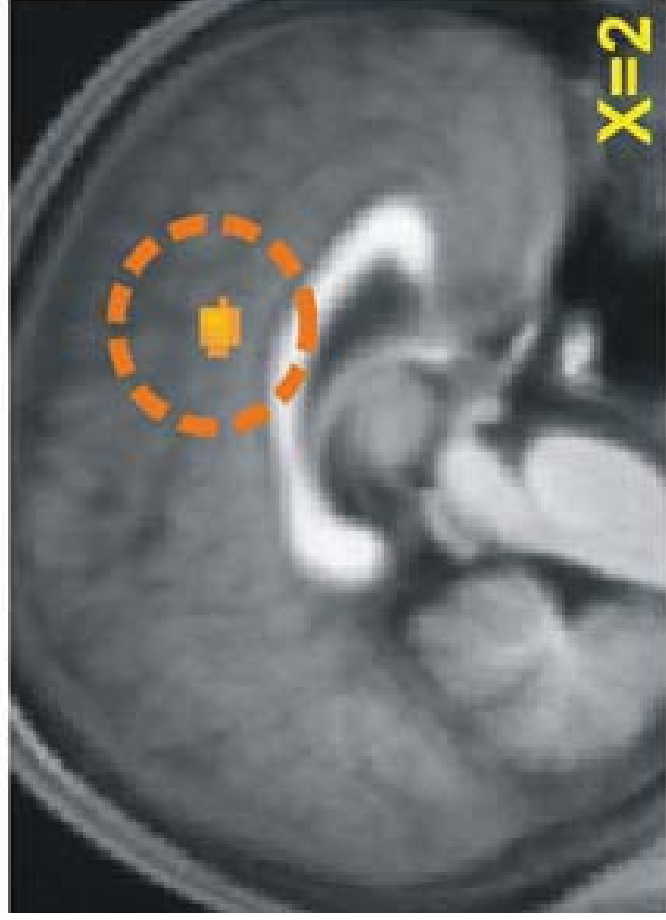
A**B**

A Correlation between anxiety ratings when the real and rubber hand is threatened

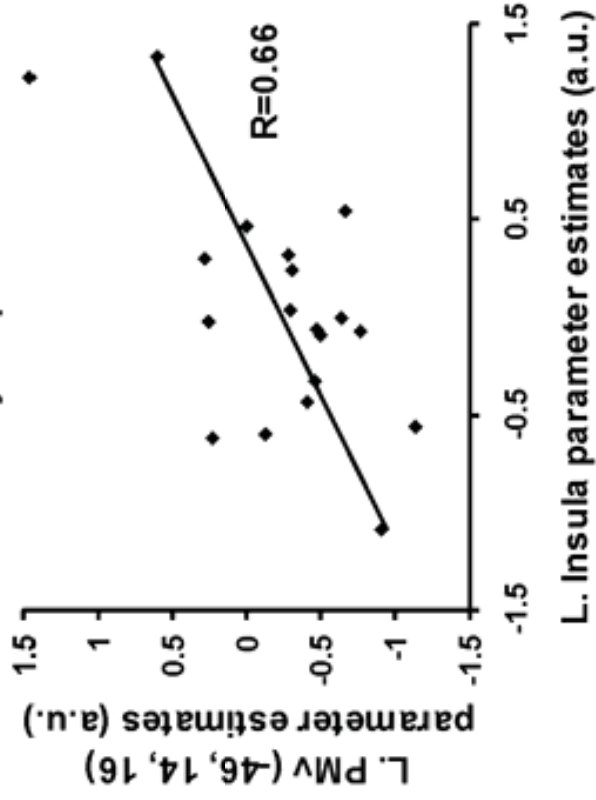


B Correlation between vividness and anxiety

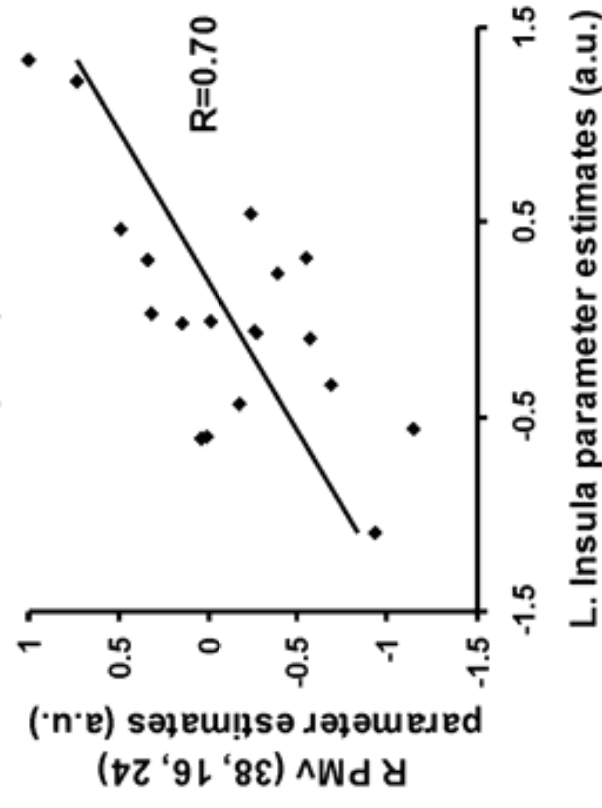




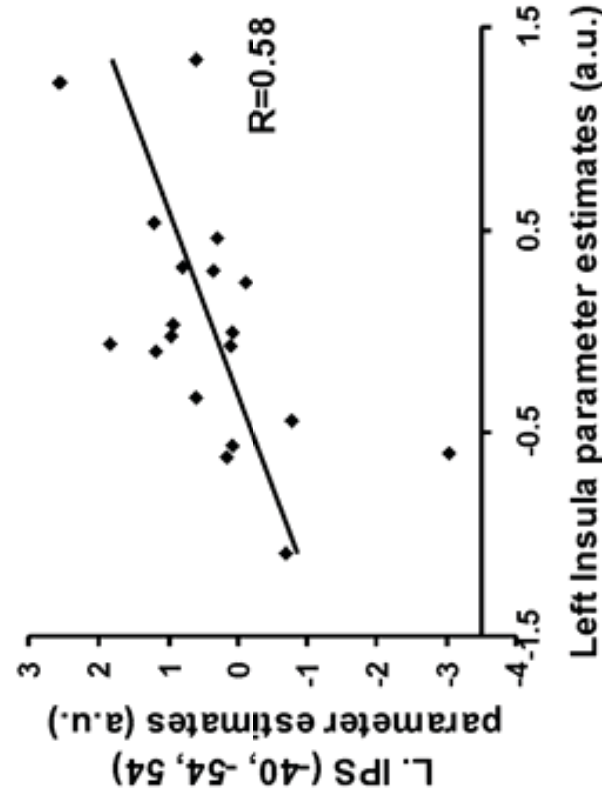
Correlation between illusion activity and anxiety response



Correlation between illusion activity and anxiety response



Correlation between illusion activity and anxiety response



Correlation between illusion activity and anxiety response

